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HEAT SHOCK PROTEINS AND THE TREATMENT OF TUMOURS

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HEAT SHOCK PROTEINS AND THE TREATMENT OF TUMOURS

The invention relates to expression vectors which express heat shock proteins or chaperones.

Conventionally, solid tumours are treated by a combination of surgery, chemotherapy and radiotherapy. Other neoplasms, such as leukaemia, are also treated by chemotherapy and radiotherapy. However, these methods of treatment are not ideal because radiotherapy and chemotherapy cause unpleasant side effects and some solid neoplasms, such as brain tumours, cannot be treated surgically. For these reasons new methods for treating and preventing tumours are constantly being sought. One method which is currently being investigated is the use of gene transfer of, for example, IL-2 or TNF encoding genes.

Tumours may be induced in mice by administering tumour cell lines. In particular, mice to which the murine macrophage tumour cell line J774 is administered develop large, intraabdominal, highly malignant lymphoreticular neoplasms. Surprisingly, it has been found that, when such tumour cells are transfected with the gene encoding the Mycobacterium leprae 65kD hsp and subsequently administered to mice, there is a decrease in or loss of tumourigenicity of the cells. Furthermore, when such transfected cells are administered to a host, such as a mouse, and the host is subsequently challenged with tumour cells from the same cell line which have not been transfected, no tumour develops. Therefore the technique has potential for an alternative or additional approach to the treatment of neoplasms.

The present invention provides a mammalian cell or

an expression vector for use as a therapeutic agent, for example in the treatment of neoplasms comprising nucleic acid including at least one sequence which encodes in expressible form a polypeptide which is a heat shock polypeptide (hsp) or a chaperone.

A polypeptide includes, for example, a heat shock protein, a heat shock protein fragment, a heat shock protein analogue, a chaperone, a chaperone fragment or a chaperone analogue. A fragment will be at least 10, preferably at least 15, for example, 20, 25, 30, 40, 50 or 60 amino acid residues in length. An analogue will be generally at least 70%, preferably at least 80% or 90% and more preferably at least 95% homologous to the heat shock protein or chaperone over a region of at least 5, preferably at least 10, for example 20, 40, 60 or 100 or more contiguous amino acids. The amino acid sequence of an analogue may differ from that of the heat shock protein or chaperone when at least one residue is deleted, inserted or substituted.

A chaperone is a protein which mediates the folding of proteins into their active conformation. Polypeptides exist which are both chaperones and heat shock polypeptides. Heat shock polypeptides, such as hsp60, hsp65 and hsp70 are involved in the folding and translocation of other proteins, and are therefore also chaperones.

Fragments include polypeptides which are not glycosylated but which retain their anti-neoplasm activity. Analogues include proteins which have been engineered with a different amino acid sequence, but which retain their anti-neoplasm activity.

The nucleic acid of the expression vector of the present invention may be double or single stranded and may be RNA or DNA. Generally the nucleic acid includes only one sequence which encodes a polypeptide as defined herein, but the nucleic acid may include 2, 3, 4 or more sequences which separately encode a polypeptide as defined herein.

The nucleic acid comprised in the expression vector or cell line of the present invention encodes any suitable polypeptide as defined herein but preferably does not encode a polypeptide of the host. Preferably the polypeptide is a chaparonin and most preferably the polypeptide is bacterial hsp, such as Mycobacterium leprae 65kD hsp or Mycobacterium leprae 70 kD hsp.

The expression vector of the invention is generally a virus, plasmid, cosmid, episomal vector or naked nucleic acid. Generally the naked nucleic acid comprises the sequence encoding the polypeptide together with regulatory sequences. The naked nucleic acid generally comprises at least 100, for example at least 200, 400, 600, 800, 1000, 2000 or 4000 base pairs. The naked nucleic acid may be single or double stranded and may be DNA or RNA. Suitable plasmids include, for example, pZIPNeo, pLXSN and MFG.

Viruses which are conveniently used as the expression vector of the invention include viruses in which the nucleic acid of the vector is RNA, for example disabled retroviruses, and DNA viruses such as the vaccinia virus or an adenovirus. The vector or virus may be administered directly to the host, so as to achieve expression of the polypeptide in infected cells, or may first be incorporated into a cell line which is

then administered to the host. When the vector or virus is administered directly to the host, the virus used is generally helper-free and replication-defective.

Preferably the expression vector comprises recombinant nucleic acid.

The present invention also provides a process for producing an expression vector other than naked nucleic acid, which process comprises cloning into a vector a gene encoding a polypeptide as defined herein. According to the process of the present invention the polypeptide-encoding gene may be cloned directly into an expression vector using known methods (Silva, Palacios, Colston and Lowrie, Microbiol Pathogenesis 12, 27-38 (1992)). The invention also provides a process for producing a cell which process comprises inserting heterologous DNA into the genome of the cell or transfecting an expression vector of the invention into a cell line. According to the present invention the heterologous DNA may be inserted into the genome using known methods (Molecular Cloning: A Laboratory Manual, T. Maniatis et al, Cold Spring Harbour, 1989).

Preferably a transfected cell line is prepared by cloning the gene encoding the polypeptide into a suitable vector, such as a retroviral shuttle vector, for example pZIPNeoSV(x), using conventional techniques and transfecting the vector into a cell line using known methods (Silva et al).

Cell lines into which expression vectors according to the present invention may be incorporated are generally mammalian and include neoplasm cell lines, MHC I and II negative cell lines, and murine and human cell lines. It is possible to use cell lines, for instance neoplasm cell lines,

that originate from the host or patient to which they will be administered, particularly in order to treat established tumours. Most preferably, the cell line which is transfected is that of the neoplasm against which protection is sought.

The expression vector or cell of the invention may further include a sequence which encodes a neoplasm associated antigen or an interleukin. The expression vector or cell of the invention may include an expression vector having a sequence which encodes a neoplasm associated antigen or an interleukin. Several studies have shown that transfection of murine tumour cells with genes encoding cytokines results in the immunological rejection of the parent tumour. The cytokine genes which have been used for this type of experiment include IL-4, IL-2, γ IFN, TNFα, IL-6, IL-7 and GM-CSF. One or more of these genes may act synergistically with a gene encoding a polypeptide of the invention and hence maximise the chances of achieving tumour eradication.

Neoplasms caused by different and unrelated neoplasm cell lines may have common neoplasm associated antigens, in which case immunisation according to the invention with one cell line which expresses such a common antigen may give protection against other neoplasms which also have common antigens (but is unlikely to give much protection against those neoplasms which do not). Accordingly, the present invention also provides a cell line which expresses a polypeptide as defined herein and a neoplasm associated antigen. Such cell lines may be transfected with an expression vector according to the invention together with a separate vector encoding a neoplasm associated antigen or they may be transfected with an

expression vector of the invention which also includes a sequence which encodes a neoplasm associated antigen in expressible form.

According to the present invention, the expression vector or cell line may be used for the prophylaxis of neoplasms in a healthy host (patient) and for the therapeutic treatment of existing neoplasms in a host (patient). The expression vector may be administered directly to the patient. When the expression vector is a virus, cell transfection may be carried out in vivo, by administering the virus directly to the host. Alternatively, in vitro immunisation may be carried out by exposing suitable lymphocytes removed from the host (patient) to a transfected cell line of the invention and returning the lymphocytes to the host (patient).

The present invention also provides (a) a method of treatment of neoplasms which comprises administering to the host an effective non-toxic amount of the expression vector or cell line, (b) the expression vector or cell line for use in the treatment of neoplasms and (c) the use of the expression vector or cell line for the manufacture of a medicament for the treatment neoplasms.

The present invention further provides the use of nucleic acid as defined herein for the manufacture of a medicament for the treatment of neoplasms.

The present invention provides an expression vector or cell as defined herein in purified form. The invention further provides an expression vector or cell as defined herein when isolated. The present invention further provides an expression vector as defined herein consisting essentially of

nucleic acid including at least one sequence which encodes in expressible form a polypeptide which is a heat shock polypeptide (hsp) or a chaperone.

The expression vector or cells according to the invention may be administered to mammals including humans by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient and the type of treatment envisaged. Thus, existing neoplasms may be treated systematically or by a route selected to deliver the vector or cells directly to the site of the lesion. Prophylactic treatment will be aimed at stimulating protective immunity in tissues likely to be affected by the target neoplasms.

The expression vector of the invention may be administered to the patient alone or in combination with liposomes or other delivery molecules. The effective dosage rate depends on whether a delivery molecule is used but is generally from 100 to 500 mg vector per gram of tumour, preferably injected locally into the tumour, or 10 to 100 mg per kilogram body weight per week injected intravenously to treat or prevent metastasis.

The active ingredient in the following formulations is a vector or cell according to the invention as defined above or an <u>in vitro</u> immunised cell.

For each of the above-indicated utilities and

indications the amount required of the individual active ingredients will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician. In general, however, for each of these utilities and indications, when therapy is to be carried out with transfected tumour cells, a suitable, effective dose will be in the range 10^4 to 10^{10} cells per kilogram body weight of recipient per week, preferably in the range 10^5 to 10^8 cells per kilogram body weight per week and most preferably in the range 10^5 to 10^7 cells per kilogram body weight per week. The dose may, if desired, by presented as two, three, four or more sub-doses administered at appropriate intervals throughout the week. When therapy is carried out with a vector containing the hsp65 gene, the effective dose will be in the range 10^4 to 10^{12} helper-free, replication-defective virus per kg body weight per week, preferably in the range 10^5 to 10^{11} virus per kg body weight per week and most preferably in the range 10^6 to 10^{10} virus per kg body weight per week. The dosage rates stated herein are applicable to each of the dosage rates stated above.

While it is possible for the compounds to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof, for example, liposomes. Suitable liposomes

include, for example, those comprising the positively charged lipid N[1-(2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA), those comprising dioleoylphosphatidylethanolamine (DOPE), and those comprising 3β[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol).

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine

the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrate (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide desired release profile.

The formulations may be applied as a topical ointment or cream containing the active ingredient in an amount of, for example, 0.075 to 20% w/w, preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in

the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatis and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening

agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Examples of neoplasms which can be treated by the expression vector or cell of the invention are, for instance, sarcomas, including osteogenic and soft tissue sarcomas, carcinomas, e.g., breast-, lung-, bladder-, thyroid-, prostate-, colon-, rectum-, pancreas-, stomach-, liver-, uterine-, and ovarian carcinoma, lymphomas, including Hodgkin and non-Hodgkin lymphomas, neuroblastoma, melanoma,

myeloma, Wilms tumor, and leukemias, including acute lymphoblastic leukaemia and acute myeloblastic leukaemia, gliomas and retinoblastomas.

In the accompanying drawings, Figure 1(a) shows the expression of Mycobacterium leprae 65kD heat shock protein (hsp) encoding gene in control and transfected cells prepared according to the invention. Figure 1(b) shows the growth of parent cells, parent cells transfected with vector alone and parent cells transfected with Mycobacterium Leprae 65 kd hsp gene over 21 days.

Figure 2 shows the in vitro cytotoxicity of splenocytes of mice immunised according to the invention. Target cells were J774-hsp 65 (□), J774 (Δ), Pu518 (◇) and Wehi 164 (O).

Figure 3 shows number of proliferating cells against DNA content. Results are shown for J774 cells, J774 cells transfected with vector alone, J774 cells transfected with hsp-65 gene by calcium phosphate precipitation and J774 cells transfected with hsp-65 gene by liposome mediated gene transfer.

Figure 4 shows percentage of cells expressing p53 epitopes in J774-hsp 65 cells.

Figure 5 shows tumour size in mice receiving plasmid containing the hsp 65 gene (O) and in mice not receiving the hsp 65 gene (□).

The invention is illustrated by means of the following examples.

EXAMPLE 1

The effect of transfecting a tumour cell line with a gene which encodes for a heat shock polypeptide (hsp) was investigated by transfecting the murine macrophage tumour cell line J774 with the Mycobacterium leprae 65kD hsp - encoding gene.

The gene transfection was carried out as described in Silva, Palacios, Colston & Lowrie, Microbiol Pathogenesis 12, 27-38 (1992). The M.leprae 65kD hsp gene was cloned into the retroviral shuttle vector pZIPNeoSV(x), which was transfected by calcium phosphate precipitation into the virus-packaging cell line psi-CRE. Supernatants from neomycin resistant clones were incubated with J774-G8 cells in the presence of polybrene and neomycin resistant cells selected. Expression of the 65kD hsp gene was assessed by FACS analysis of cells following indirect labelling using monoclonal antibody C1118, which recognises the M.leprae 65kD protein, and fluorescein isothiocyanate labelled rabbit antimouse $F(ab')_2$. Figure 1(a) shows the expression of hsp65 in control and transfected cells as assessed by indirect immunofluorescence. The broken line represents reactivity with the second antibody alone.

The tumourigenicity of the parent (J774) and transfected cell lines was tested in euthymic and athymic mice. Tumour cells were either the parent J774-G8 (J774) cells, the parent cell line transfected with vector alone (J774-vector), or the parent cell line transfected with the mycrobacterial hsp65 gene (J774-hsp65). Successful, stable transfection was further confirmed

by Northern and Western blotting as described in Silva, et al. Five mice per group were injected intraperitoneally with 10^6 cells. Figure 1(b) shows the growth of the cell lines in normal Balb/c and athymic Balb/c mice respectively. Tumour size was calculated by weighing the primary tumour and abdominal organs of test mice and subtracting the weight of abdominal organs of five age-matched normal controls.

It is clear from the results shown in Figure 1 that the incidence of tumours in mice injected with J774-hsp65 cells is much lower than that in mice injected with J774 or J774-vector cells. 21 days after injection all mice injected with J774 or J774 vector cells have tumours weighing at least 8g, whereas the mice injected with J774-hsp65 cells have either not developed tumours, or have tumours weighing less than 3g.

EXAMPLE 2

Normal Balb/c, Balb/c athymic and CBA mice were injected intraperitoneally with the number of tumour cells indicated in Table 1. The cells were first resuspended in 0.5ml endotoxin free PBS. Tumour cells were prepared in the same way as in Example 1 and were either the parent cell line (J774), the parent cell line transfected with the mycobacterial hsp65 gene (J774-hsp65), or the parent cell line transfected with the vector alone (J774-vector). Tumour incidence was determined by autopsy and histological examination 21 days after injection of tumour cells. Results are shown in Table 1.

TABLE 1

The tumourigenicity of transfected and parent reticulum sarcoma cells in Balb/c, Balb/c athymic and CBA mice

Injected Tumour Cells		Tumour incidence Balb/c	Balb/c (athymic)	CBA
	10^5	4/5	-	-
J774	10^6	9/10	5/5	0/10
	10^7	5/5	-	-
J774-	10^5	3/5	-	-
vector	10^6	14/15	5/5	-
	10^6	0/10	0/10	0/10
J774-hsp65	10^7	1/10	-	-
	$5x10^7$	0/5	-	-

EXAMPLE 3

J774-hsp65 cells were prepared according to the method described in Example 1. Normal and athymic Balb/c mice were immunised with four intraperitoneal injections of 10^6 J774-hsp65 cells given at weekly intervals. On day 28 the mice were challenged intraperitoneally with 10^6 reticulum sarcoma cells of differing origins. Tumour incidence and tumour size were recorded 21 days after challenge. Tumour size was determined in the same way as in Example 1. Results are given in Table 2.

TABLE 2

The effect of immunisation with transfected tumour cells (J774-hsp65) on subsequent challenge with reticulum sarcoma cells

Immunisation	Challenge	Tumour incidence		Tumour Size (g)	
		Balb/c (normal)	Balb/c (athymic)	Balb/c (normal)	Balb/c (athymic)
J774-hsp65	J774	0/10	5/5	0.28±0.24(p<0.001)	9.99±0.79
None	J774	9/10	5/5	10.20±1.18	10.28±0.33
J774-hsp65	J774-vector	0/15	5/5	0.68±0.40(p<0.001)	9.81±1.09
None	J774-vector	10/10	5/5	10.93±0.35	9.92±0.38
J774-hsp65	Pu518	2/10	5/5	3.33±1.06(p<0.001)	8.09±0.57
None	Pu518	5/5	4/4	9.96±0.50	7.69±0.58
J774-hsp65	Wehi 164	7/9	5/5	7.74±1.12	7.14±1.04
None	Wehi 164	5/5	4/4	8.80±0.68	7.87±0.58

The results given in Table 2 show that normal Balb/c mice, when injected with J774-hsp cells, develop good immunity against J774 and J774-vector cells. The results also show that normal Balb/c immunised mice have some resistance to the Pu518 cell line; not all mice developed tumours and in those which did, the tumours which formed were of significantly smaller size than in non-immunised mice. Resistance of immunised mice against the Wehi 164 cell line was poor; a large proportion of mice developed tumours which were similar size to the tumours which developed in non-immunised mice.

Immunisation with J774-hsp65 cells had no effect on Balb/c athymic mice.

EXAMPLE 4

J774-hsp65 cells were prepared according to the method described in Example 1.

The in vitro cytotoxic activity of splenocytes from mice immunised with J774-hsp65 cells was investigated by immunising normal Balb/c and Balb/c athymic mice with four intraperitoneal injections of 10^6 J774-hsp65 cells at weekly intervals. On day 28, spleens were removed and single cell suspensions prepared in RPMI 1640 plus 10% foetal calf serum. The cells were incubated for 6 days with J774-hsp65 cells irradiated by 30 Gy. B cells were removed by panning on rabbit anti-mouse IgG-coated plates. CD4 and CD8 T cell subsets were purified using the method described in Lukacs and Kurlander, J. Immunol.

143, 3731-3736 (1989). NK and CD4 or CD8 cells were removed by treatment of 10^7/ml cells with 10 μg/ml PK136 (anti-NK1.1) and anti-L3T4 or anti-Lyt 2 monoclonal antibodies at 4°C for 30 min, followed by incubation with 1/10 dilution of rabbit complement at 37°C for 60 min, resulting in CD4 and CD8 populations which were over 90% pure as determined by FACS analysis. Cytotoxic activity was measured by incubating varying number of CD4 or CD8 effector cells with 2000/well target cells labelled with ^{51}Cr in triplicate wells for 6 h at 37°C in a v-bottomed 96-well microtiter plate. ^{51}Cr release was measured in wells containing effector T cells and target cells ($cpm_{experimental}$), wells containing target cells incubated in medium alone ($cpm_{spontaneous}$) and in wells containing target cells plus 0.1% Triton X ($cpm_{maximal}$). Cytotoxicity was calculated using the formula:

$$\% \text{ cytotoxicity} = \frac{(cpm_{experimental}) - (cpm_{spontaneous})}{(cpm_{maximal}) - (cpm_{spontaneous})} \times 100$$

The results are shown in Figure 2 as the mean ± standard deviation. Target cells were J774-hsp65 (□), J774 (Δ), Pu518 (◇) and Wehi 164 (○).

The results in Figure 2 show that <u>in vitro</u> cytotoxicity of splenocytes from immunised mice is as great against J774-hsp65 as against J774 cells and much greater than against Pu 518 cells (cytotoxicity was significant but less) and Wehi 164 cells (no cytotoxicity).

EXAMPLE 5

The effect of hsp-transfection on the cell cycle of tumour cells was examined by staining the cells with a DNA-specific benzimide dye, Hoechst 33342. J774 tumour cells transfected with the Mycobacterium leprae hsp-65 gene by two different methods, Ca-phosphate precipitation (J774-HSP-Ca) and liposome mediated gene transfer (J774-HSP-L), as well as untransfected and vector-transfected control J774 cells (2 x 10^6/ml) were stained with 10 μg/ml Hoechst dye for 30 min. at 37 C. Using FACS analyser, DNA histograms were obtained, and the percentage of cells in each phase of the cell cycle was determined.

Results in Figure 3 show that the number of proliferating cells with high DNA content (S and G2 phases) decreased in tumour cells transfected with the hsp-65 gene (J774-HSP-Ca and J774-HSP-L). Simultaneously, an increase has been found in the number of hsp65 expressing cells with low DNA content (G0/G1 phases) compared to the control tumour cells. These experiments indicate that transfection of tumour cells with hsp-65 gene results in a change in the cell cycle control, and suggest that one of the basic characteristics of tumour cells, uncontrolled proliferation, is altered.

EXAMPLE 6

Mutations in the p53 gene resulting in dysfunctional p53 tumour suppressor protein is the most frequently observed genetic

lesion in malignant tumours. Tumour cells transfected with Mycobacterium leprae hsp65 and control tumour cells were examined for the expression of p53 tumour suppressor protein. J774-HSP cells were prepared according to the method described in Example 1. Expression of p53 protein was assessed by FACS analysis. Cell membranes were permeabilized by 0.05% saponin, then the cells were incubated with monoclonal antibodies specific for p53 (Ab-248 and Ab-421, provided by D.Lane) and FITC-labelled rabbit antimouse F(ab')2 second antibody.

Figure 4 shows increased expression of both p53 epitopes in J774-hsp65 cells. Since p53 is a DNA-binding protein essential for normal cell cycle control, our experiments suggest that hsp65 may exert its effect on the cell cycle through p53 tumour suppressor protein. The increased chaperone activity of the hsp65 transfected cells could result in the proper folding and conformation of the ineffective, mutant p53 protein, thereby correcting its loss of tumour suppressor function.

EXAMPLE 7

Balb/c mice with advanced histiocytic sarcomas received in vivo gene therapy with the Mycobacterium leprae hsp65 gene fourteen days after tumour induction with 10^6 J774 tumour cells. A mixture of 100 μg pZIPML65 plasmid and 100μg liposome was injected four times intraperitoneally into the tumour and intravenously to treat metastatic cells. On day 21 mice were sacrificed, autopsy performed and tumour sizes were determined.

Results in Figure 5 show tumour regression in mice receiving plasmid containing the hsp65 gene. Only one tumour was found in the group of six treated mice, while all six untreated controls developed large tumours by day 21. Although further studies are needed and histological evidence obtained for tumour regression, our data indicate that the hsp65 gene can be successfully delivered in vivo into tumours resulting in tumour regression. In vivo injection of the hsp65 gene can result in lost tumorigenicity of those cells which become transfected while the cytotoxic T cell response generated by the transfected cells could complete the eradication of remaining untransfected cells. This duel effect makes hsp65 an attractive choice in the gene therapy of cancers.

CLAIMS

1. An expression vector for use in the treatment of neoplasms which vector comprises nucleic acid including at least one sequence which encodes in expressible form a polypeptide which is a heat shock polypeptide (hsp) or a chaperone.

2. An expression vector according to claim 1 wherein the polypeptide is a chaparonin.

3. An expression vector according to claim 1 or claim 2 wherein the polypeptide is bacterial hsp.

4. An expression vector according to claim 3 wherein the hsp is Mycobacterium leprae 65kD hsp or Mycobacterium leprae 70kD hsp.

5. An expression vector comprising nucleic acid including a sequence which encodes in expressible form a heat shock polypeptide other than an expression vector comprising nucleic acid including a sequence which encodes Mycobacterium leprae 65 kD hsp, Mycobacterium leprae 70kD hsp or 65kDa antigen of Mycobacterium tuberculosis.

6. An expression vector according to any one of claims 1 to 5 which is a virus.

7. An expression vector according to claim 6 wherein the nucleic acid is RNA.

8. An expression vector according to claim 7 which is a disabled retro virus.

9. An expression vector according to claim 7 which is a retroviral shuttle vector.

10. An expression vector according to claim 9

which is pZIPNeoSV(x).

11. An expression vector according to claim 6 which is vaccinia virus.

12. An expression vector according to claim 6 which is an adenovirus.

13. An expression vector according to any one of claims 1 to 5 which is a plasmid.

14. An expression vector according to any one of claims 1 to 5 which is an episomal vector.

15. An expression vector according to any one of the preceding claims which further includes a sequence which encodes a neoplasm associated antigen.

16. An expression vector according to any one of the preceding claims which further includes a sequence which encodes an interleukin.

17. A cell which includes an expression vector as claimed in any one of the preceding claims.

18. A neoplasm cell line the cells of which include an expression vector as claimed in any one of claims 1 to 16.

19. A human cell or cell line according to claim 17 or 18.

20. A cell or virus having in its genome heterologous nucleic acid encoding a polypeptide as defined in claim 1 in expressible form.

21. A cell or cell line according to any one of claims 17 to 20 which further includes an expression vector having a sequence which encodes a neoplasm associated antigen.

22. An expression vector according to any one of

claims 6 to 12 which further includes an expression vector having a sequence which encodes a neoplasm associated antigen or an interleukin.

23. A pharmaceutical composition comprising the expression vector, cell, cell line or virus as claimed in any one of the preceding claims and a pharmaceutically acceptable carrier.

24. A pharmaceutical composition according to claim 23 wherein the carrier is a liposome.

25. A pharmaceutical composition according to claim 23 or 24 which further comprises an expression vector having a sequence which encodes a neoplasm associated antigen or an interleukin.

26. A pharmaceutical composition comprising an effective non-toxic amount of the expression vector, cell line, cell or virus as claimed in any one of claims 1 to 22.

27. A process for producing an expression vector as claimed in any one of claims 1 to 16, which process comprises cloning into a vector a gene encoding the polypeptide.

28. A process for producing a cell or cell line as claimed in any one of claims 17 to 21 which process comprises cloning into a vector a gene encoding the polypeptide and transfecting the vector into a cell or cell line.

29. Use of an expression vector, cell line, cell or virus according to any one of claims 1 to 22 for the manufacture of a medicament for the treatment of neoplasms.

30. Use of nucleic acid as defined in any one of claims 1 to 4 for the manufacture of a medicament for the

treatment of neoplasms.

31. Use according to claim 29 or 30 wherein the treatment is of existing neoplasms.

32. Use according to claim 29 or 30 wherein the treatment is prophylactic.

33. A method of treatment of neoplasms which comprises administering to a host an effective non-toxic amount of an expression vector, cell line or virus as claimed in any one of claims 1 to 22 or a nucleic acid as defined in any one of claims 1 to 4.

34. A method of treatment according to claim 33 wherein the host is healthy and the treatment is prophylactic.

35. A method of treatment according to claim 33 wherein the host has a neoplasm and the treatment is therapeutic.

36. An expression vector for use as a therapeutic agent which comprises nucleic acid including at least one sequence which encodes in expressible form a polypeptide which is a heat shock polypeptide (hsp) or a chaperone.

Fig.1(a).

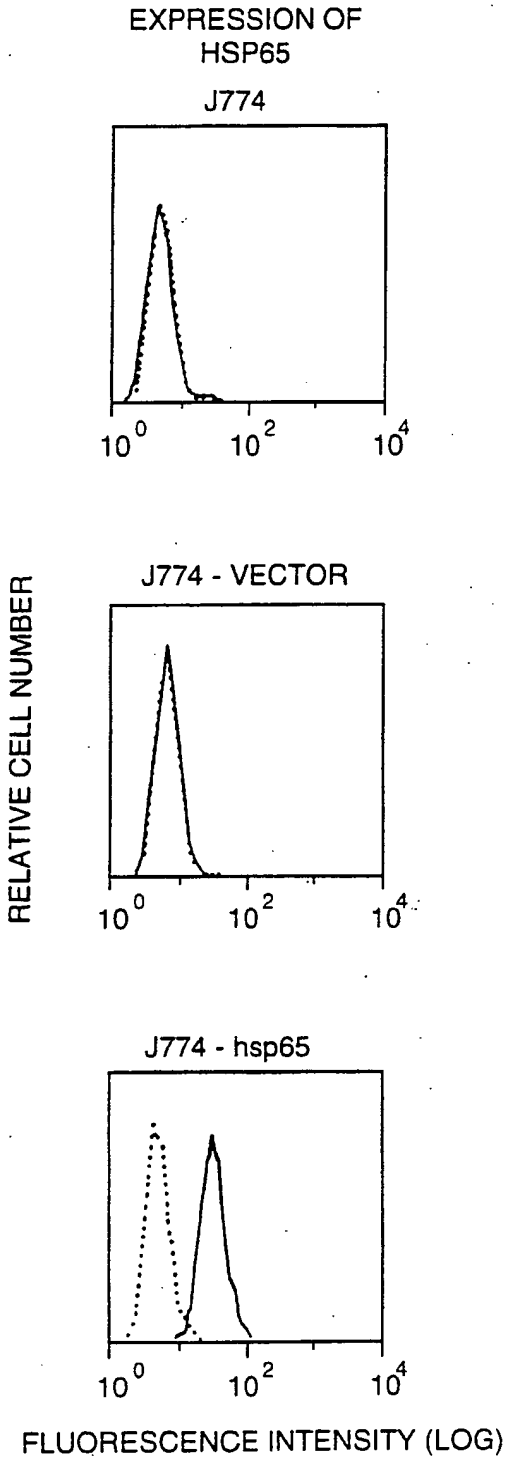

EXPRESSION OF HSP65
J774
10^0
10^2
10^4
J774 - VECTOR
10^0
10^2
10^4
RELATIVE CELL NUMBER
J774 - hsp65
10^0
10^2
10^4
FLUORESCENCE INTENSITY (LOG)

Fig.1(b)

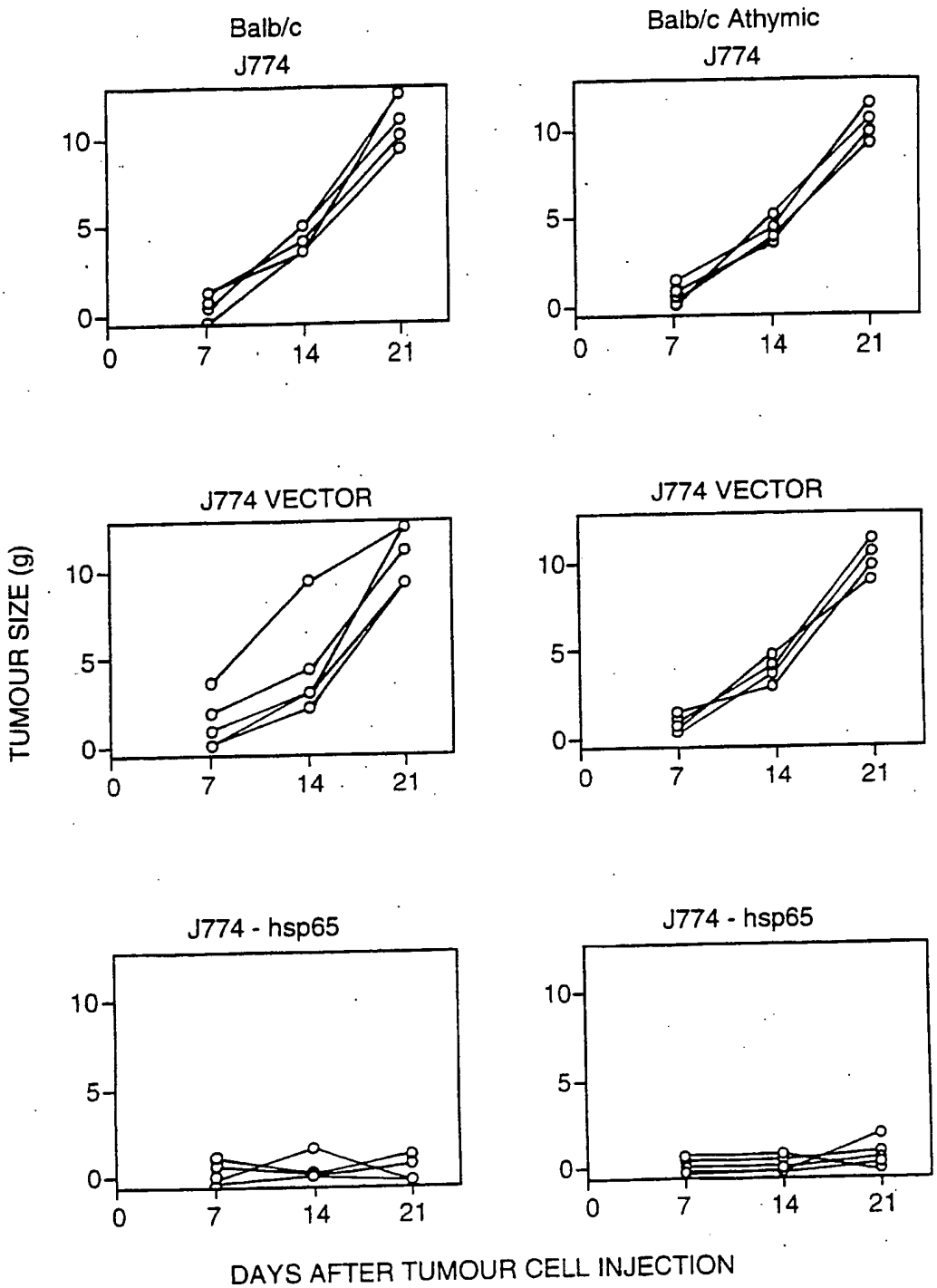

GROWTH OF TUMOURS IN MICE
Balb/c
J774
Balb/c Athymic
J774
J774 VECTOR
J774 VECTOR
J774 - hsp65
J774 - hsp65
TUMOUR SIZE (g)
0
5
10
0
7
14
21
DAYS AFTER TUMOUR CELL INJECTION

Fig.2

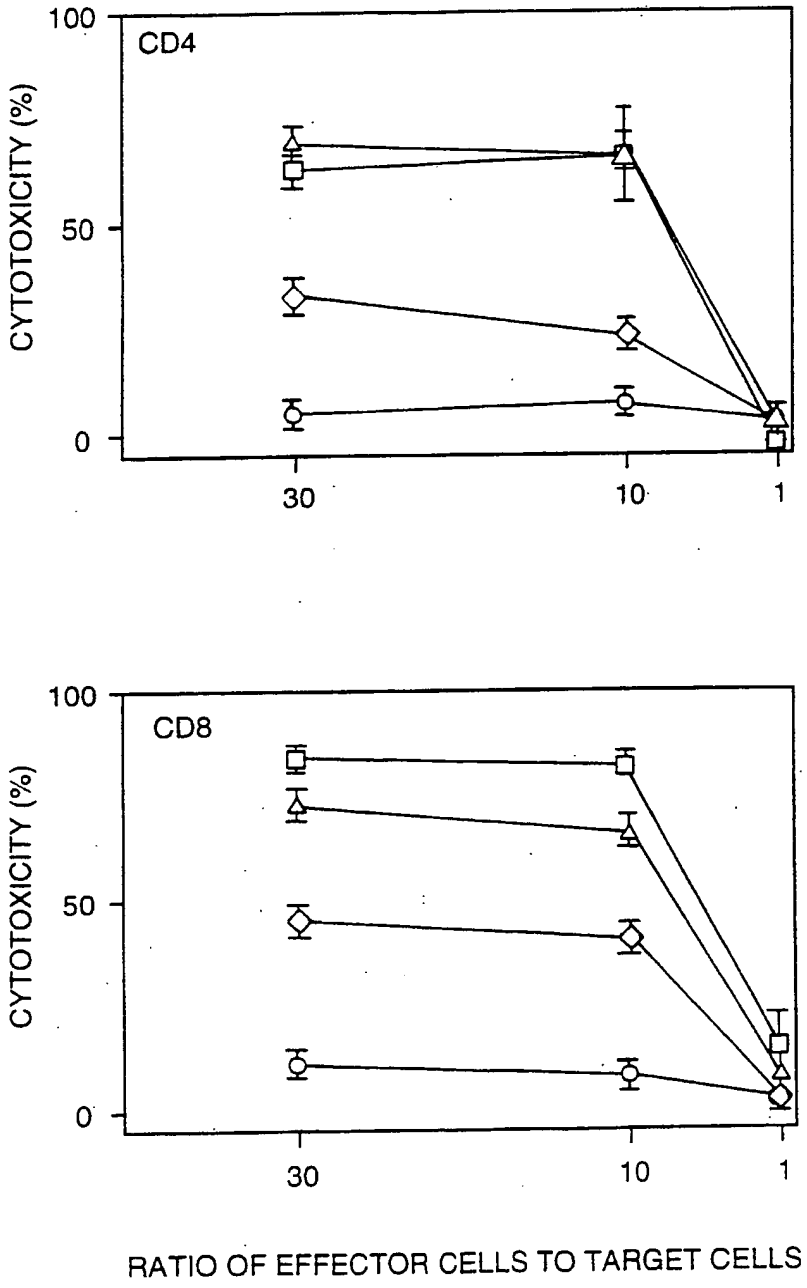
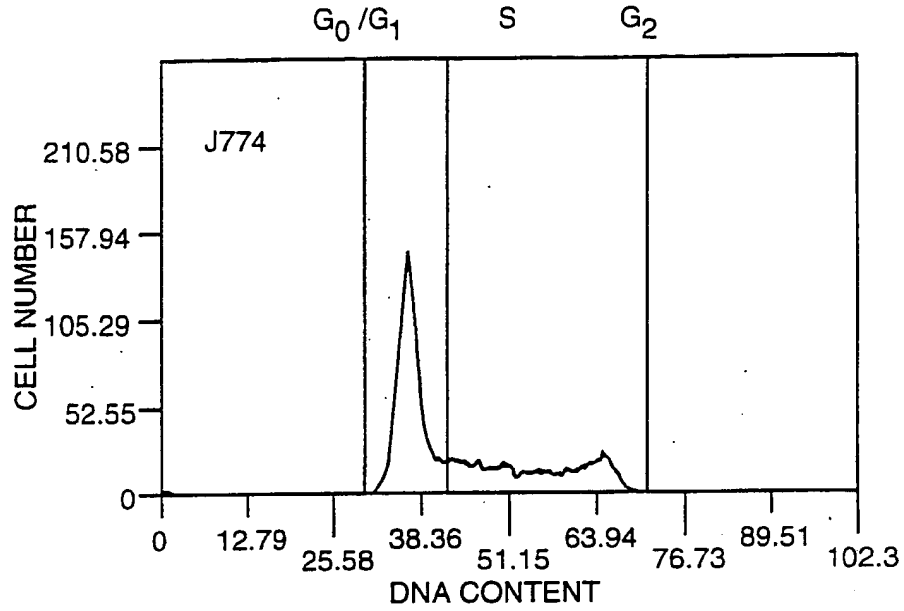

CD4
CD8
CYTOTOXICITY (%)
100
50
0
30
10
1
RATIO OF EFFECTOR CELLS TO TARGET CELLS

Fig.3.


G0 /G1
S
G2
J774
210.58
157.94
105.29
52.55
0
CELL NUMBER
0
12.79
25.58
38.36
51.15
63.94
76.73
89.51
102.3
DNA CONTENT

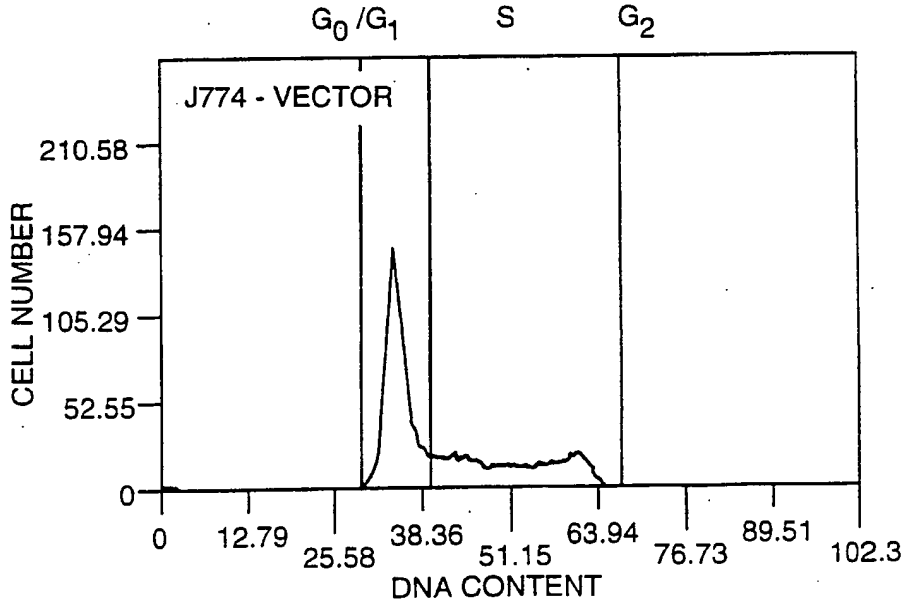
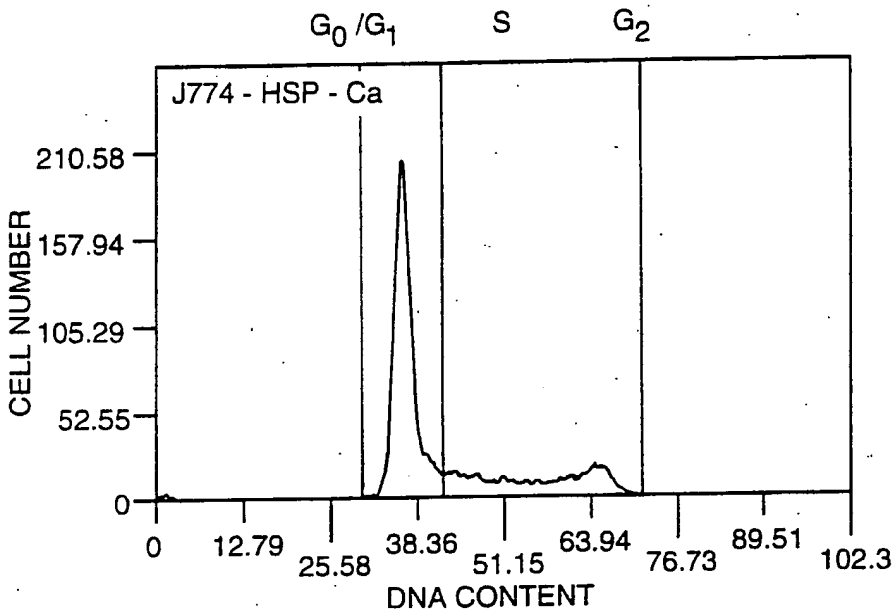

G0 /G1
S
G2
J774 - VECTOR
210.58
157.94
105.29
52.55
0
CELL NUMBER
0
12.79
25.58
38.36
51.15
63.94
76.73
89.51
102.3
DNA CONTENT

Fig.3 (Cont.)


G0 /G1
S
G2
J774 - HSP - Ca
210.58
157.94
105.29
52.55
0
CELL NUMBER
0
12.79
25.58
38.36
51.15
63.94
76.73
89.51
102.3
DNA CONTENT

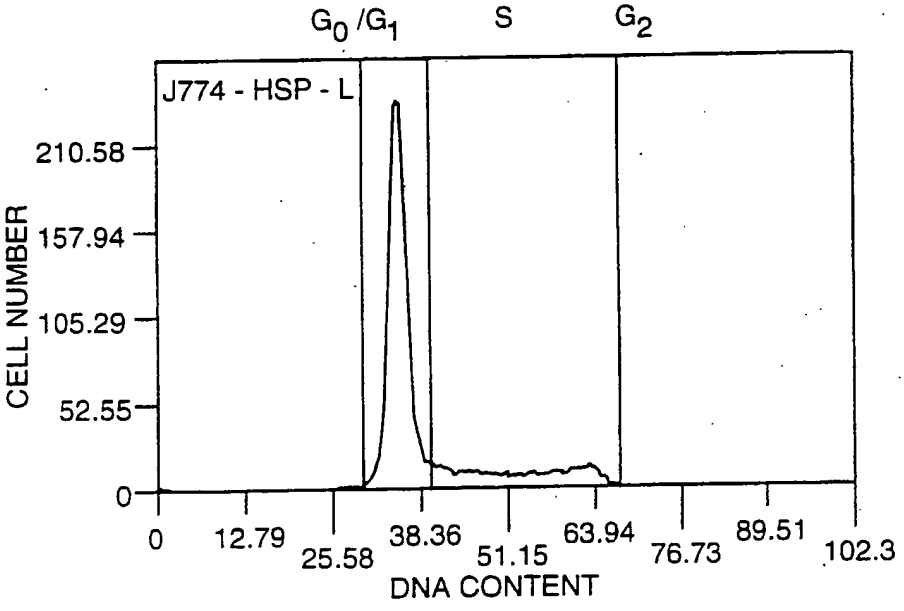

G0 /G1
S
G2
J774 - HSP - L
210.58
157.94
105.29
52.55
0
CELL NUMBER
0
12.79
25.58
38.36
51.15
63.94
76.73
89.51
102.3
DNA CONTENT

Fig.4

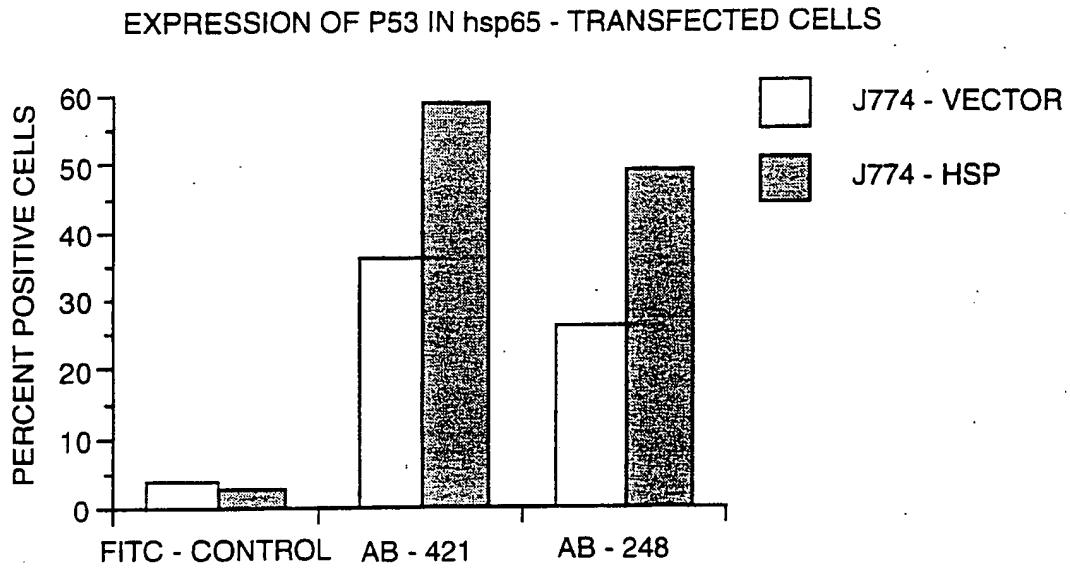
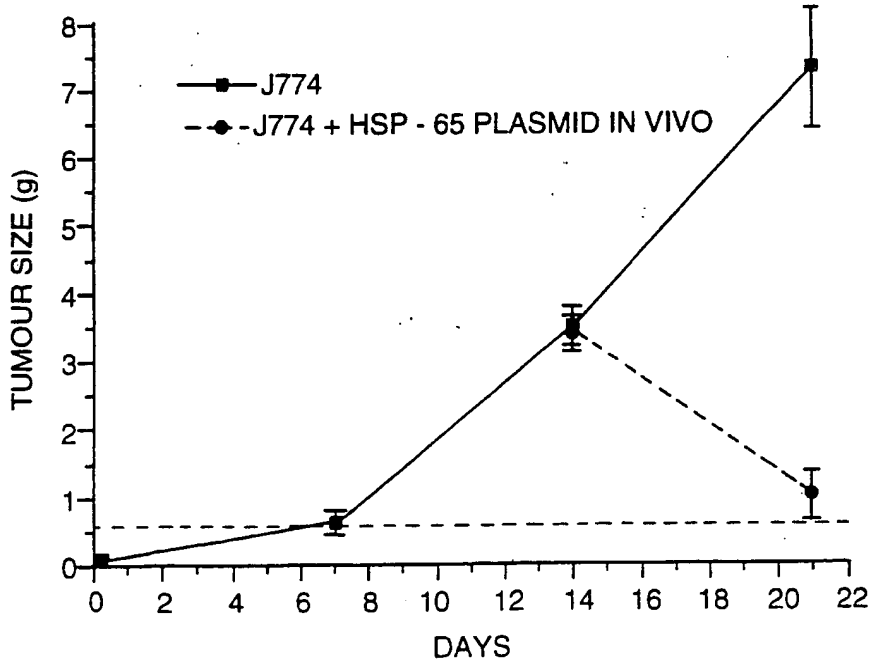

EXPRESSION OF P53 IN hsp65 - TRANSFECTED CELLS
PERCENT POSITIVE CELLS
60
50
40
30
20
10
0
FITC - CONTROL
AB - 421
AB - 248
J774 - VECTOR
J774 - HSP

Fig.5


J774
J774 + HSP - 65 PLASMID IN VIVO
TUMOUR SIZE (g)
8
7
6
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18 20 22
DAYS

INTERNATIONAL SEARCH REPORT

Interna. Application No
PCT/GB 93/02339

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/31 C12N15/86 C12N5/10 A61K37/02 A61K31/70
//A61K9/127,C12N15/24,C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF LEUKOCYTE BIOLOGY vol. 0, no. 2 , 1991 , NEW YORK, US page 68 LOWRIE, D. ET AL. 'Non-MHC-restricted specific recognition by T cells of J774 cells expressing transfected Mycobacterium leprae HSP65'	1-4, 6-10, 17-20, 27,28, 33-36
Y	see abstract 194 & 28th National Meeting of the SLB and 21st Leucocyte Culture Conference, Snowmass at Aspen, Colorado September 28-October 1, 1991	1-14

	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 1994	28 -02- 1994
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna J Application No
PCT/GB 93/02339

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MICROBIAL PATHOGENESIS vol. 12, no. 1 , January 1992 , ABERDEEN, GB pages 27 - 38 SILVA, C. ET AL. 'Mycobacterium leprae 65hsp antigen expressed from a retroviral vector in a macrophage cell line is presented to T cells in association with MHC class II in addition to MHC class I' see the whole document	1-4, 6-10,17, 20,27, 28,33-36
Y		1-18, 20-22, 29-35
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 83, no. 10 , May 1986 , WASHINGTON US pages 3121 - 3125 ULLRICH, S. ET AL. 'A mouse tumor-specific transplantation antigen is a heat shock-related protein' see abstract see page 3124, left column, paragraph 1	1-10,15, 17,18, 20-22, 29-35
Y	SCIENCE vol. 254 , 1 November 1991 , LANCASTER, PA US pages 713 - 716 GOLUMBEK, P. ET AL. 'Treatment of established renal cancer by tumor cells engineered to secrete Interleukin-4' see the whole document	16,22
X	IMMUNOLOGY TODAY vol. 10, no. 7 , July 1989 , CAMBRIDGE GB pages 218 - 221 WATSON, J.D. 'Leprosy: understanding protective immunity' see the whole document	5,6,11, 13,17, 20,27, 28,36
Y		1-14
P,X	JOURNAL OF EXPERIMENTAL MEDICINE vol. 178 , July 1993 pages 343 - 348 LUKACS, K. ET AL. 'Tumor cells transfected with a bacterial heat-shock gene lose tumorigenicity and induce protection against tumors' see the whole document	1-4, 6-10,17, 18,20, 27-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB93/02339

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 33-35 are directed to a method of treatment of (di (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.